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Short-Term and Long-Term Intra-Individual Variations and Critical Differences of Coagulation Parameters

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Summary: The intra-individual variations of 9 coagulation parameters were studied during short-term (within-one-day) and long-term (six months) periods. Two groups of healthy individuals (viz. 60 and 274 persons) were involved. Moreover, critical differences have been calculated from the total variations, comprising both biological and analytical variations. The influences of external factors such as sex, smoking and the use of oral contraceptives have also been studied.

The variability and critical differences found in coagulation parameters in this study appeared to be of the same order as those observed in parameters usually determined in clinical chemistry and haematology. The application of critical differences in the evaluation and use of laboratory data, also in coagulation studies, enhances the objectivity of the judgement. Moreover, in the follow-up of patients the sensitivity of the parameters is increased.

Kurz- und langzeitige intra-individuelle Änderungen sowie kritische Differenzen von Gerinnungskenngrößen

Zusammenfassung: Für 9 Gerinnungskenngrößen wurden innerhalb eines Tages und über einen Zeitraum von 6 Monaten die intra-individuellen Variationen bestimmt. Dazu wurden 2 Gruppen von gesunden Individuen (bestehend aus 60 beziehungsweise 274 Personen) ausgewählt.

Aus den Gesamtvariationen, sowohl die biologischen wie auch die analytischen Variationen umfassend, wurden die kritischen Differenzen errechnet. Einflußgrößen wie Geschlecht, Rauchen und Einnahme von oralen Kontrazeptiva wurden dabei in Betracht gezogen.

Die Variabilität und die kritischen Differenzen der untersuchten Gerinnungskenngrößen lagen in der gleichen Größenordnung wie die der üblichen klinisch-chemischen und haematologischen Laboratoriumskenngrößen.

Die Anwendung der kritischen Differenzen für die Evaluation und richtige Interpretation der Laborbefunde erhöht auch bei Gerinnungsuntersuchungen die Objektivität der Beurteilung.

Außerdem wird bei longitudinaler Betrachtungsweise der Patienten die Sensitivität der untersuchten Kenngrößen erhöht.

Introduction

Because of the complexity of the process of coagulation under physiological and pathological circumstances, proper interpretation of values of clotting parameters is difficult, especially if longitudinal comparisons are required. Usually, the patient's results are compared with the so-called "transverse reference values", which are obtained from blood samples in an apparently healthy population. The analytical data for a parameter in one individual are compared with the 95 percentile or $\bar{x} \pm 2$ SD-range in the reference group. The comparison is concentrated on inter-individual variation (1, 2, 3). The members of the "healthy" reference population frequently consist of selected groups such as donors from the blood transfusion service, laboratory personnel or students. Evidently, this is not an ideal situation, because several selection mechanisms may be present. It seems therefore to be of advantage to take each individual as his own reference in clinical studies.

However, for evaluation of serial changes in a patient's laboratory results, knowledge of physiological fluctuations of values in healthy individuals is required.

More than 25 years ago *R. J. Williams* (4) introduced the concept of biochemical individuality and demonstrated the uniqueness of each individual in many physiological, biological and biochemical aspects. The biological variation can be divided into an intra-individual component and an inter-individual component. Different components show short-term and long-term variations, e. g. of biological and analytical origin. For the interpretation of a patient's data, the variation components should be known in order to detect possible pathological conditions, i. e. variations within a certain period exceeding the usual physiological ones. From the biological and the analytical variation the so-called critical difference can be calculated (5). The purpose of the critical difference is to indicate the range which covers the majority (95%) of the differences between two subsequently obtained values of one laboratory parameter in one healthy individual. When the observed differences exceed the critical difference we consider them to be of clinical importance.

A real problem is that in the field of coagulation parameters, sufficient data about intra-individual variation in time are generally not available. Reliable longitudinal appreciation of such data is therefore generally impossible.

The purpose of the present study is to assess the intra-individual variation of three screening tests and six specific coagulation tests and to calculate the critical differences in a large population of healthy volunteers during one day and during six months. To minimize analytical and pre-analytical influences special attention was paid to the constancy of analytical procedures and the pre-analytical treatment of the blood samples.

Persons, Materials and Methods

Individuals

The investigation of the within-one-day intra-individual variation was started with a group of 60 apparently healthy volunteers in the age range 18 – 53 years. The group contained 23 males and 37 females from hospital laboratory personnel (20 persons), medical staff (16) and students (24 persons). A group of 300 volunteers participated in the investigation of the six-month intra-individual variation. In the first two months 21 persons withdrew for various reasons (inconvenience, departure from the area, etc.). During the six-month period another five persons were removed from the study on account of apparent disease. As a result, the group of individuals participating in the study consisted of 274 volunteers in the age range 18 to 63 years. Some were workers in various branches of chemical industry (84 persons), others were hospital laboratory personnel (82 persons) and clerical personnel (28 persons), male and female nurses (63 persons) and volunteers from a variety of other groups (17 persons). The group was subdivided into 148 males and 126 females including 72 male smokers, 54 female smokers and 61 women taking oral contraceptives.

Body weight, height and age of all individuals were registered. No gross restrictions were imposed on diet or activity during the study. The nature and purpose of the study were explained beforehand to all individuals and its design was approved by the Ethical Committee.

All individuals were judged to be healthy at the beginning of the study by medical interview; no person suffering from chronic or recurrent illness was admitted. During the studies no serious illness or injury was observed apart from haematoma after venipuncture. No drugs, apart from oral contraceptives, were involved.

Scheme of venipuncture

For the within-one-day period, all blood specimens from one individual were taken at fixed times i. e. 8.30 and 11.00 a. m. and 2.00 and 4.30 p. m. in the course of one day. These times were chosen because in normal practice about 95% of blood samples for analysis are drawn within these hours.

For the month-to-month period 274 volunteers were subjected to venipuncture monthly over a period of six months. For each volunteer the monthly venipuncture was carried out at the same time of the day.

Specimens

The individuals had a 10 to 15 minutes rest in a sitting position before venipuncture. While the subject was in a comfortable sitting posture blood was collected in vacuum citrate-containing blood collection tubes (Terumo®). Brief tourniquet pressure was released immediately before venipuncture. Platelet free plasma samples were obtained by twenty minutes centrifugation of the tubes at 20 000 *g* in order to prevent the fracture of residual platelets by deep freezing. The plasma samples of all individuals were frozen at −78 °C until the day of analysis, when all specimens of one individual were thawed at room temperature, mixed thoroughly and analysed in the same run.

The overall analytical variation, given as coefficient of correlation (CV), can therefore be considered as an intra-assay variation. Storage influences were excluded by handling control samples of a separate group of blood donors in the same way as the individuals who participated in the study. The influence of freezing and thawing as well as storage at −78 °C appeared to be almost negligible.

Analytical procedures

The automated analyses were the same as those in daily routine use for the analysis of patient's specimens (tab. 1).

Variance components

In general three variance components can be distinguished, a biological, an analytical and an "other one" (tab. 2) (5). For the present investigation the "other" component can be neglected; the main part of this component is usually specimen collection, and to minimize this component, blood collection is standardised in this study by using a vacuum collecting system. For each individual, total individual variances (s_T^2) can be calculated from the observations. Assuming the analytical component to be known, the biological one can be calculated from:

$$s_B^2 = s_T^2 - s_A^2$$

In this study we investigated the intra-individual variation.

Moreover, all samples from one individual were analysed in the same run (no inter-run variance). According to the terminology of table 2, $s_B = s_P$ and $s_A = s_S$ and the reported results are mainly given in coefficients of variation (percentages). For instance $CV_B = s_B/\bar{x} \cdot 100\%$ whereas the mean $\bar{x}$ is based on the observations of one individual.

Tab. 1. Methods and intra-assay precision of the coagulation parameters.

Parameter	Method	Reagent	Instrumentation	CV* %
Activated partial thromboplastin time	Coagulation	Merz and Dade	Schnitger and Gross coagulometer	3.3
Calcium thromboplastin time	Coagulation	Own reagent from human brain	Schnitger and Gross coagulometer	3.4
Thrombin time	Coagulation	Merz and Dade	Schnitger and Gross coagulometer	5.0
Fibrinogen	Coagulation (Clauss)	Merz and Dade	Schnitger and Gross coagulometer	3.1
Factor V	Coagulation	Boehringer Mannheim	Schnitger and Gross coagulometer	3.5
Factor X	Amidolytic	Kabi Vitrum	Cobas Bio	2.2
Antithrombin III	Amidolytic	Kabi Vitrum	Schnitger and Gross coagulometer	2.1
α_2-Antiplasmin	Amidolytic	Kabi Vitrum	Schnitger and Gross coagulometer	2.0
Plasminogen	Amidolytic	Kabi Vitrum	Schnitger and Gross coagulometer	2.0

* Intra-assay coefficient of correlation

Tab. 2. Symbols of the variance components used in this study.

S_T^2 = total variance of one individual from a reference population
$S_T^2 = S_B^2 + S_A^2 + S_O^2$

S_B^2 = biological variance

$S_B^2 = S_P^2 + S_I^2$ S_P^2 = intra individual variance
S_I^2 = inter-individual variance
S_I^2 = absent in this study

$S_A^2 = S_S^2 + S_L^2$ S_S^2 = variance within the run
S_L^2 = variable between runs
S_L^2 = absent in this study

S_O^2 = "Other" variance, e.g. specimen collection

Corresponding coefficients of variation (percentages) are denoted by CV_T, CV_B and CV_A.

d_K = critical difference

$d_K = 2\sqrt{2S_T^2} = 2\sqrt{2(S_P^2 + S_S^2)}$

or

$d_K = 2\sqrt{2CV_T^2} = 2\sqrt{2(CV_P^2 + CV_S^2)}$

To characterize the variability of CV_P for each parameter, three characteristics of the histogram of 60 and 274 CV_P values will be reported in the tables:

- the percentage of individuals with $CV_T > CV_S$ (denoted by n_{var}).
- the median value, denoted by CV_{P50}.
- the ninety percentile value, denoted by CV_{P90}.

Critical differences

The critical difference d_K has been developed as a tool to follow the course of one laboratory parameter in one individual in consecutive measurements. The critical difference is dependent on the total variance s_T^2 for the one individual concerned. Because in our study the critical difference is predominantly dependent on the intra-individual variance s_P^2 and the within-run variance s_S^2, it can be written as:

$$d_K = 2\sqrt{2s_T^2} = 2\sqrt{2(s_P^2 + s_S^2)} \quad \text{(in units)}$$

or

$$d_K = 2\sqrt{2(CV_P^2 + CV_S^2)} \quad \text{(in percentages)}$$

When longitudinal investigations are performed in clinical laboratory practice, the analytical variance s_A^2 is composed of the intra-run variance s_S^2 and the inter-run variance s_L^2; the critical difference is then calculated as:

$$d_K = 2\sqrt{2(s_P^2 + s_A^2)} \quad \text{(in units)}$$

or

$$d_K = 2\sqrt{2(CV_P^2 + CV_A^2} \quad \text{(in percentages)}$$

Statistical methods

Each parameter in this study has been investigated with the *Friedman* rank test (6), in order to ascertain whether a systematic pattern could be found. Such a pattern could be either an upward or a downward trend or a systematic low or high value at one particular point. Several two group comparisons (like male – versus female, smokers/non smokers) were performed using the *Mann-Whitney* test (6). Correlations were studied using the *Spearman* rank correlation test (6).

Results

Analytical variation

The analytical within-run variations (CV) are presented in terms of coefficients of variation (tab. 1), which are always under 5%. The clotting assays vary from 3.1% to 5.0%, whereas the amidolytic tests show a small range from 2.0% to 2.2%.

Within-one-day intra-individual variation (short-term variation)

In order to investigate whether trends and/or systematic patterns existed in the consecutive parameter values during the day, the *Friedman* rank test (6) was applied. Except for a slightly increasing trend during the day in the fibrinogen concentration and a decrease in the activated partial thromboplastin time, no trends were found. Moreover, no patterns could be found over the day although plasminogen showed significantly higher *Friedman* rank values at 8.30 a. m.

Intra-individual variations were calculated in samples taken at different times of one day. These were considered to be due to the total physiological variability. The results of CV_{P50}, d_{K50}, CV_{P90} and d_{K90} are given in table 3. They are based upon the fact that either $CV_T > CV_P$, in which case CV_P is calculated, or $CV_T < CV$ and CV_P cannot be calculated and is set to zero. Moreover, the percentage of individuals in the group in whom $CV_T > CV_S$, is expressed as n_{var}. In four tests (antithrombin III, α_2-antiplasmin, plasminogen and Factor X) considerable biological variability in more than 85% of participating individuals was found ($n_{var} > 85\%$). Intermediate variability (n_{var} 51%–85%) was seen in three tests (calcium thromboplastin time, activated partial thromboplastin time and fibrinogen), while least variability was found ($n_{var} < 50\%$) in two other tests (Factor V and thrombin time). The within-one-day intra-individual variation CV_{P50} and CV_{P90} were greater than zero for all plasma coagulation constituents except for the CV_{P50} of thrombin time and Factor V.

Tab. 3. Intra-individual variations and critical differences during one day.

n_{var} = percentage of individuals with $CV_T > CV_S$
CV_{P50} = median intra-individual coefficient of variation
CV_{P90} = 90 percentile of intra-individual coefficient of variation
d_{K50} = critical difference based on CV_{P50}
d_{K90} = critical difference based on CV_{P90}

Analyte	n_{var} %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Calcium thromboplastin time	60	0.9	9.9	7.8	24.1
Activated partial thromboplastine time	77	3.4	13.4	9.7	29.0
Thrombin time	28	0.0	14.1	4.0	18.1
Fibrinogen	75	3.5	13.2	13.0	37.8
Factor V	28	0.0	9.9	3.9	14.8
Antithrombin III	95	5.2	15.9	16.3	46.5
α_2-Antiplasmin	92	6.6	19.5	16.4	46.7
Plasminogen	92	3.8	12.1	15.4	43.9
Factor X	88	4.8	14.9	13.7	39.2

As shown in table 3 the intra-individual variations ranged from 0.0%–6.6% (CV_{P50}) and from 3.9% – 16.4% (CV_{P90}). The critical differences varied from 9.9% to 19.5% (d_{K50}) and from 14.8% to 46.7% (d_{K90}).

Applying the *Mann-Whitney* test (6) to the values, significant differences for the intra-individual variations of plasminogen between the male and female groups are found, which seems to be of clinical importance (tab. 4). No differences between the sexes in intra-individual variation are found for the other parameters. Additionally, no significant differences in intra-individual variations are found between the male smokers/non smokers, the female smokers/non smokers groups and the female groups using/not using oral contraceptives.

Tab. 4. Significant differences in the intra-individual variations and critical differences during one day between males and females ($P < 0.05$).
n = number of individuals.

n_{var} = percentage of individuals with $CV_T > CV_S$
CV_{P50} = median intra-individual coefficient of variation
CV_{P90} = 90 percentile of intra-individual coefficient of variation
d_{K50} = critical difference based on CV_{P50}
d_{K90} = critical difference based on CV_{P90}

Analyte	n	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Plasminogen					
♂	23	3.0	10.2	11.2	32.2
♀	37	4.3	13.4	17.9	50.9

Month-to-month intra-individual variation (long-term variation)

To follow the course of a chronic disease or for the purpose of monitoring healthy individuals in a preventive medicine setting, we investigated month-to-month variations over a period of six months. The intra-individual variations were calculated from the results of the six-month period. The *Friedman* rank test (6) was applied to every individual's coagulation data. No systematic low or high values or trends were found.

The results for CV_P are summarized in table 5. Month-to-month intra-individual variations, CV_{P50} and CV_{P90}, were all significantly different from zero for all constituents except for the CV_{P50} of thrombin time, where $CV_T < CV$. As shown in table 5, the intra-individual variations (CV_{P50} and CV_{P90}) varied from 0.0% – 10.0% and from 5.8% – 17.8%. The critical differences (d_{K50} and d_{K90}) varied from 10.6% to 29.6% and from 19.6% to 50.7%.

Differences related to sex were found for calcium thromboplastin time, thrombin time, Factor V, antithrombin III, α_2-antiplasmin, plasminogen and Factor X, although these differences do not seem to be clinically relevant. Only the plasminogen levels between females using or not using oral contraceptives show a difference, which seems to be clinically important (tab. 6).

Scatter-diagrams and coefficients of correlation showed no relationship between intra-individual variations and age, height, body weight and levels of corresponding laboratory parameters.

Tab. 5. Intra-individual variations and critical differences during six months in 274 persons.

n_{var} = percentage of individuals with $CV_T > CV_S$
CV_{P50} = median intra-individual coefficient of variation
CV_{P90} = 90 percentile of intra-individual coefficient of variation
d_{K50} = critical difference based on CV_{P50}
d_{K90} = critical difference based on CV_{P90}

Analyte	n_{var} %	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Calcium thromboplastin time	92	5.8	19.0	11.6	34.2
Activated partial thromboplastine time	93	6.8	21.4	12.7	37.2
Thrombin time	39	0.0	14.1	5.8	21.7
Fibrinogen	100	10.0	29.6	18.9	54.2
Factor V	77	3.6	14.2	8.5	26.0
Antithrombin III	90	3.1	10.6	6.6	19.6
α_2-Antiplasmin	97	5.8	17.4	13.4	38.3
Plasminogen	98	7.7	22.5	17.8	50.7
Factor X	98	5.9	17.8	11.8	40.0

Tab. 6. Significant differences in the intra-individual variations and critical differences during six months between females using or not using oral contraceptives ($P < 0.05$).
n = number of individuals

n_{var} = percentage of individuals with $CV_T > CV_S$
CV_{P50} = median intra-individual coefficient of variation
CV_{P90} = 90 percentile of intra-individual coefficient of variation
d_{K50} = critical difference based on CV_{P50}
d_{K90} = critical difference based on CV_{P90}

Analyte	n	CV_{P50} %	d_{K50} %	CV_{P90} %	d_{K90} %
Plasminogen					
– using oral contraceptives	61	11.5	33.0	21.8	61.9
– Not using oral contraceptives	57	7.3	21.4	17.7	50.4

Discussion and Conclusions

Comparison of our results with data from the literature in the field of coagulation was hardly possible, because no attention has been paid in the literature to the issues of the variability, the intra-individual variation and the critical difference. Recently *Costongs* et al. (7, 8, 9) published such data for clinical chemistry and haematology parameters.

The coagulation parameters investigated in this study can be divided into two parts: three screening tests and six specific tests. The analytical variations in both groups are in the same range as in a series of clinical chemistry and haematology parameters reported earlier (7, 8, 9). Therefore, the mentioned clotting parameters are in this respect comparable with other laboratory data.

The variability of the measured parameters, both within one day and during six months, is surprisingly low for the thrombin time and Factor V. This phenomenon was hardly ever seen in clinical chemistry and haematology parameters (8, 9). The other parameters do not differ significantly from these data. As expected, the long-term CV_{P90} is higher than the short-term CV_{P50}. This is not the case for antithrombin III, α_2-antiplasmin and Factor X. Especially for antithrombin III, the long-term CV_{P90} is much smaller than the short-term one. Variations over the day should therefore be interpreted carefully. The interpretability of laboratory tests depends on the d_{K90}. For an objective judgement of the clinical significance of changes in subsequent laboratory values of a laboratory parameter within one individual, consideration of d_{K90} is essential. The d_{K90} data obtained for the clotting tests are found at intermediate levels. The highest levels in the long-term have been observed for fibrinogen (54%). This is e. g. comparable with the values found in the determination of serum urea (d_{K90} = 53.7%) in clinical chemistry and with the total leukocyte count in haematology (d_{K90} = 53.8%). The lowest long-term d_{K90} value was calculated for antithrombin III (19.6%), being comparable with serum albumin (d_{K90} = 18.3%) in clinical chemistry and with erythrocyte determination in haematology (d_{K90} = 17.8%).

Thus, on the basis of intra-individual variation and critical difference, the d_{K90} values for the coagulation parameters in this study fall in the same range as other clinical chemical and haematological laboratory parameters with respect to their clinical interpretation. Determination of coagulation parameters is apparently no less reliable for clinical use than the traditional laboratory parameters in clinical chemistry and haematology.

Data on intra-individual variations and critical differences of coagulation parameters and other laboratory parameters can facilitate decisions on diagnosis and (further) treatment of disease.

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